METHOD AND DEVICE FOR ENRICHING AND DETECTING MICROORGANISMS IN A BIOLOGICAL SAMPLE

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII FILE

[0001] This application is being filed electronically via EFS-Web and includes an electronically submitted Sequence Listing in .txt format. The Sequence Listing written in file NSequence-US-EU-A.txt, created Jan. 26, 2021, 1,401 bytes, is hereby incorporated herein by reference in its entirety and for all purposes.

FIELD OF INVENTION

[0002] The present invention relates to the technical field of detection of microorganisms; particularly, to a method and device for enriching and detecting microorganisms in a biological sample by reducing the interference from human-derived nucleated cells in the biological sample.

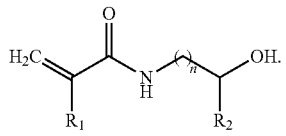
DESCRIPTION OF THE RELATED ART

[0003] When using molecular detection methods to detect pathogenic microorganisms (including bacteria, mycoplasmas, fungi, viruses, spores etc.) in biological samples, the cell count and genome size of human cells in the samples are much larger than the pathogenic microorganisms, and the amount of human DNAs is usually tens of thousands or even millions of times that of DNAs of pathogenic microorganisms. Therefore, during the process of molecular detection of pathogenic microorganisms, the background interference of human DNAs has always been a major challenge. Currently, there are methods for differential lysis, such as QIAamp DNA Microbiome Kit and the recently published modified method (Nanopore metagenomic enables rapid clinical diagnosis of bacterial lower respiratory infection. Charalampous et al., Nature Biotechnology, 2019); as well as methylation modification methods to remove human DNAs, such as NEBNext® Microbiome DNA Enrichment (New England Biolabs). However, these methods have distinct disadvantages of complicated operations and inconsistent effects.

SUMMARY OF THE INVENTION

[0004] The present invention is to provide a method and device for enriching and detecting microorganisms in a biological sample by reducing the interference from human-derived nucleated cells such as leukocytes in the biological sample.

[0005] The present invention provides a method for enriching and detecting microorganisms in a biological sample, which includes the following steps: a) collecting the biological sample; b) filtering the sample through Sterile Acrodisc® White Blood Cell Syringe Filter (PALL) or a polymer-modified substrate, human-derived nucleated cells in the sample are captured or separated while the microorganisms in the sample pass or flow through the filter or polymer-modified substrate into filtrate; and c) detecting the microorganisms present in the filtrate. The nucleated cells include one or more of erythroblasts, leukocytes and cancer cells. The polymer is prepared by the polymerization of one or more monomers having the structure of the formula (1):



(1)

[0006] In formula (1), R_1 is independently selected from the group consisting of hydrogen, methyl, ethyl, hydroxyl, C_{1-12} alkyl, phenyl; R_2 is independently selected from the group consisting of hydrogen, methyl, ethyl, C_{1-6} alkyl, amino, phenyl; and n is an integer of 1 to 5.

[0007] Preferably, the microorganisms in the biological sample are bacteria.

[0008] Preferably, the microorganisms in the biological sample are fungi.

[0009] Preferably, the human-derived nucleated cells are leukocytes.

[0010] In some embodiments, the retention rate of the microorganisms in the filtrate is above 65%.

[0011] In some embodiments, the retention a of the microorganisms in the filtrate is above 80%.

[0012] In some embodiments, the erythrocytes can pass or flow through the polymer-modified substrate into the filtrate, and the retention rate of the erythrocytes is above 80%.

[0013] In some embodiments, the platelets can pass or flow through the polymer-modified substrate into the filtrate, and the retention rate of the platelets is above 80%.

[0014] In some embodiments, the fibrinogens can pass or flow through the polymer-modified substrate into the filtrate, and the retention rate of the fibrinogens is above 80%.

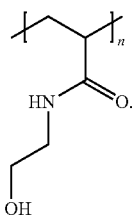
[0015] In some embodiments, the detection rate of the microorganisms in the filtrate is 2 fold higher than the samples without filtration.

[0016] In some embodiments, the detection rate of the microorganisms in the filtrate is 40 fold higher than the sample without filtration.

[0017] In some embodiments, the monomer of formula (1) comprises N-Hydroxyethyl acrylamide, N-(2-Hydroxyethyl) acrylamide, NHEMAA, and N-(2-Hydroxyethyl) acrylamide, HEAA.

[0018] In some embodiments, the polymer further comprises an additional monomer, which may be butyl methacrylate, and the monomer of formula (1) is copolymerized with described additional monomer to form a copolymer.

[0019] In some embodiments, the polymer has the structure of formula (2):



(2)

[0020] In formula (2), n is an integer of 10 to 50.